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RENAL COCCIDIOSIS IN INTERIOR CANADA GEESE, *BRANTA CANADENSIS INTERIOR* TODD, OF THE MISSISSIPPI VALLEY POPULATION[1]

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ABSTRACT: Kidneys from 309 Interior Canada geese from three locations in the Mississippi Flyway were examined for renal coccidia. Oocysts and/or young zygotes of *Eimeria* sp. were found in 6.8% of goose kidneys sampled. Only one type of renal coccidian oocyst was observed. Significantly more immature geese were infected than adults; however, there was no significant difference observed between the prevalences of infection in male and female birds. A host cellular response to zygotes and oocysts was noted in the majority of infected adult geese. Heavily infected kidneys were hypertrophic with minute foci on the surface of the organ. Histological examinations showed large numbers of unsporulated oocysts accumulated in distended collecting tubules, resulting in pressure necrosis to adjacent tissue and urate retention. Zygotes were observed in the cytoplasm of tubule cells and extracellularly in interstitial tissue. Infected tubule cells were characterized by the peripheral location of the nuclei, cytoplasmic basophilia, and cellular hypertrophy. This is the first report of an *Eimeria* sp. in the kidneys of Canada geese of the Mississippi Valley population.

INTRODUCTION

Renal coccidiosis in domestic and wild birds has been known to cause severe kidney damage that can ultimately result in the death of the host. Although several species of renal coccidia have been reported from wild birds (Christiansen, 1952; Walden, 1961; Wobeser, 1974; Nation and Wobeser, 1977; Greiner et al., 1981; Gajadhar et al., 1982, 1983), very little is known about the parasite's prevalence and pathology within free-flying populations of Canada geese. *Eimeria truncata* (Railliet and Lucet, 1890) is perhaps the best known renal coccidian of geese. However, most of the knowledge of the disease caused by this parasite is associated with domestic rather than wild geese.

Mortality caused by *E. truncata* in Canada geese of the South Atlantic Flyway was investigated at Pea Island National Wildlife Refuge, North Carolina (Critcher, 1950; Farr, 1954). These studies showed that renal coccidia in Canada geese of that flyway were severely pathogenic, causing or contributing to significant winter losses. Subsequent investigations of the occurrence of this protozoan parasite have indicated that distribution in wild geese was limited to the Atlantic and Pacific flyways (Hanson et al., 1957; Levine, 1973) and *E. truncata* was considered to be the only renal coccidian to infect Canada geese.

The present paper reports the prevalence and distribution of renal coccidia (*Eimeria* sp.) in Interior Canada geese of the Mississippi Valley population (MVP) and describes the pathologic effects of the parasite in this host.

MATERIALS AND METHODS

Three hundred nine Canada geese of different sex and age groups were collected from three locations in the Mississippi Flyway: Winisk, Ontario; Horicon National Wildlife Refuge (NWR), Wisconsin; and Union and Alexander counties in southern Illinois. These areas were selected because they are major staging areas of MVP Canada geese during migration.

At Winisk, Ontario, 69 hunter-killed geese

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TABLE 1. Sex and age composition and the prevalence of renal coccidia in Canada geese collected from three locations in the Mississippi Flyway, 1979–1981.

Collection site	Month and year	Immature		Adult		Total
		Males	Females	Males	Females	
Winisk, Ontario	9/1979	0/3*	0/3	0/8	0/8	0/22
Winisk, Ontario	9/1980	2/12	2/11	1/12	3/12	8/47
Horicon NWR, Wisconsin	10/1979	0/6	0/6	0/6	0/6	0/24
Horicon NWR, Wisconsin	11/1979	0/12	0/12	1/12	0/12	1/48
Horicon NWR, Wisconsin	10/1980	0/6	2/6	0/6	0/6	2/24
Horicon NWR, Wisconsin	11/1980	1/12	1/12	0/12	1/12	3/48
Union County Refuge, Illinois	2/1980	3/12	0/12	0/12	0/12	3/48
Horseshoe Lake Refuge, Illinois	2/1981	3/12	1/12	0/12	0/12	4/48
Total		9/75	6/74	2/80	4/80	21/309

* Denotes number infected birds/number examined.

were collected in early September 1979 and 1980 (Table 1). At Horicon NWR geese were taken at two different periods during each fall season. Samples were taken in early October as geese began to arrive from the breeding grounds and again in mid-November before the birds migrated farther southward. Horicon samples were obtained by cannon netting and totaled 144 geese for 1979 and 1980 combined.

The third area of sampling was located on the wintering grounds of this subpopulation in southern Illinois at Union County Refuge in 1980 and at Horseshoe Lake Refuge in 1981. Ninety-six geese were collected in late February of each collection year by using swim-in traps. Methods described by Hanson (1949, 1962) were used to determine the sex and age of the geese.

Kidneys and ureters were removed during necropsy; one kidney was placed in a whirl-pac plastic bag and frozen, the other kidney fixed in 10% formalin. Wet smears of thawed kidney tissue were examined microscopically to detect coccidian oocysts. A minimum of five smears were made from different regions of each kidney and ureter; the smears were examined using a light microscope at 140 and 1,000× magnification. Histological sections were made of the anterior, middle, and posterior portion of each fixed kidney to detect coccidian oocysts and developmental stages. Kidney tissues were sectioned at 5 μm by using standard histological procedures and stained with hematoxylin-eosin (H&E) or Giemsa stains. All developmental stages and oocysts observed in wet smears or histological sections were measured with an ocular micrometer.

To induce sporulation, large numbers of unsporulated oocysts were teased from unfrozen infected kidneys of two immature males from Union County Refuge. The oocysts were placed in Petri dishes containing a thin layer of 2.5% potassium dichromate and in dishes containing 1-day-old tap water and left at room temperature for 7 to 21 days. The oocysts were examined at 24-hr intervals.

Chi-square tests were used to determine significant differences in prevalence of infection between sex and age groups for combined sample areas. Values were considered significant at $P < 0.05$.

RESULTS

Description of oocysts and zygotes

Only one species of coccidian oocyst was found in kidneys of MVP Canada geese examined during this study. The unsporulated oocyst was characterized by its slightly compressed micropyle and rounded bottom (Fig. 1). Each of the oocysts contained a single spherical sporont, somewhat centrally located, with a residuum or single granule sometimes present inside the oocysts. The oocyst wall was smooth and composed of a single layer about 1 to 1.5 μm in width.

Mean length and width measurements of 150 oocysts taken from six infected kidneys were, respectively, 25.8 ± 1.1 μm (22.5–27.7 μm) and 18.4 ± 0.9 μm (16.6–

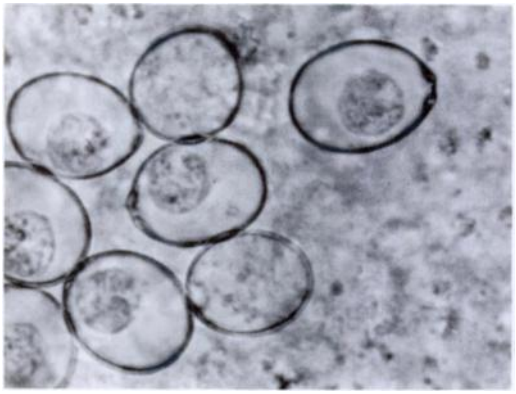

FIGURE 1. Oocysts of *Eimeria* sp. in wet smear from the kidney of an Interior Canada goose (×440).

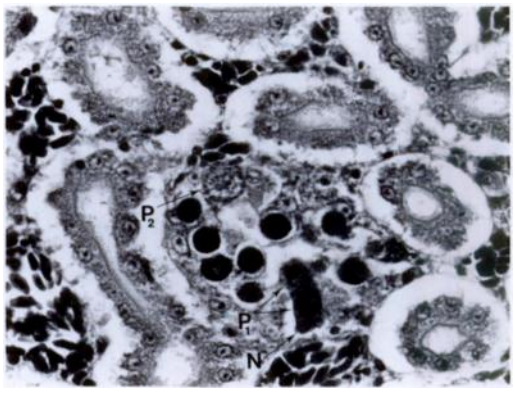

FIGURE 2. Intracellular zygotes infecting kidney tubule cells of an Interior Canada goose showing types one (P_1) and two (P_2). Note the two parasites within the cytoplasm of one tubule cell and the position of the cell nucleus (N) (H&E, ×350).

20.9 μm). The sporont had a mean diameter of 11.8 ± 1.3 μm (7.5–13.2 μm). Attempts to sporulate oocysts found in frozen and unfrozen kidney tissue were unsuccessful.

Young zygotes observed in the cytoplasm of renal tubular cells (Fig. 2) were spherical in shape and were seen more commonly in histological sections than were oocysts. Only two types of zygotes were observed. The first type consisted of many spherical subunits or granules throughout the parasite cell. These granules appeared as tightly bound solid masses of basophilic cytoplasm (Fig. 2) or the granules themselves appeared to have distinct circular definition and were distributed throughout the cellular cytoplasm. There was usually a discernible parasitophorous vacuole surrounding young forms of the parasite within the cytoplasm of infected tubular cells. However, vacuoles were not always detected around older forms of the parasite.

The second type of zygote did not have the basophilic granules distributed throughout the parasite's cytoplasm. In this type, the granules were smaller, fewer, and distinctly concentrated in the peripheral regions of the parasite (Fig. 2). These granules, possibly oocyst wall forming bodies, appeared to form a ring around the somewhat eccentric nuclei, indicative of oocyst wall formation.

When only zygotes were observed in kidney sections, identification was based on the size, shape, and appearance of the parasite observed and the host cell's response to the presence of the protozoan in its cytoplasm. These forms had a mean diameter of 12.8 ± 2.1 μm (9.4–17.2 μm, $n = 150$). Commonly both kidneys were infected with oocysts; however, when only zygotes were found in kidneys sectioned histologically, the opposite kidney from the same bird used for wet smears was usually devoid of oocysts. These intracellular forms were recognized rarely in wet smears because of the difficulty in identifying them when unstained.

Prevalence of infection

Twenty-one Canada geese, 6.8% of the total sample, harbored oocysts and/or zygotes of renal *Eimeria* sp. (Table 1). Samples taken from Winisk had the highest number and prevalence of infected geese (8/69 = 11.6%) followed by the southern Illinois (7/96 = 7.3%) and the Horicon (6/144 = 4.2%) samples. There was a significant difference ($P < 0.05$) in the prev-

FIGURE 3. Gross view of an Interior Canada goose kidney infected with *Eimeria* sp.

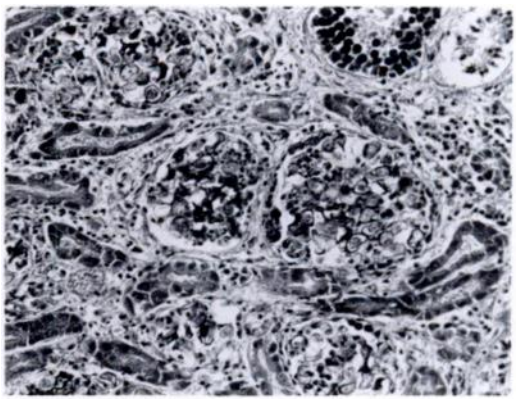

FIGURE 4. The accumulation of oocysts of *Eimeria* sp. in kidney tubules of an Interior Canada goose (H&E, ×310).

alence between immature and adult age classes infected with renal coccidia, with immature geese accounting for 71.5% of the infection and the rest adults. No significant difference was detected with respect to host sex.

Pathological effects of renal coccidia

The infection could best be described as a latent infection in the majority of the infected kidneys examined. When moderate numbers of oocysts or zygotes were seen in wet smears or sections, the gross appearance of the kidney was normal in size and color. Two immature males collected from southern Illinois, however, had kidneys which appeared abnormal on gross examination. These kidneys were enlarged and mottled with 1–3-mm whitish streaks and foci on the surface of the organ (Fig. 3). There was apparent urate retention in the kidney tissue. Large numbers of oocysts were found in wet smears and histological sections made from tissue of those kidneys. These pathologic changes were attributed to coccidiosis. No lesions were observed in any other tissues.

Unsporulated oocysts were observed primarily in the collecting tubules but were found also in interstitial tissue. In areas where large numbers of oocysts had accumulated, the tubules were markedly distended and accumulated oocysts caused rupture of the cytoplasm of adjacent tubule cells resulting in the destruction of the normal tubular architecture (Fig. 4). Moderate obstructive effects were also present proximal to these areas. Collecting ducts were often distended with material resembling partially dissolved urates as well as oocysts. There were areas where pressure necrosis indicative of acute damage were noted in the tissue, particularly near the center of kidney lobules.

Young zygotes were observed most frequently in the cytoplasm of renal epithelial cells; however, they were often present in the cytoplasm of mononuclear inflammatory cells, as well as extracellularly in the interstitial tissue. Infected tubule cells were characterized by peripheral locations of nuclei, cellular hypertrophy and cytoplasmic basophilia. Zygotes were usually seen one per cell but occasionally two parasites were observed in the cytoplasm of one tubule cell (Fig. 2). Maturation of young zygotes to oocysts was destructive and resulted in disruption of the host cell's cytoplasmic membrane. Glomeruli of the kidney were uninvolved.

A host cellular response to the intracellular and extracellular zygotes and oocysts was observed in the kidneys of adult geese.

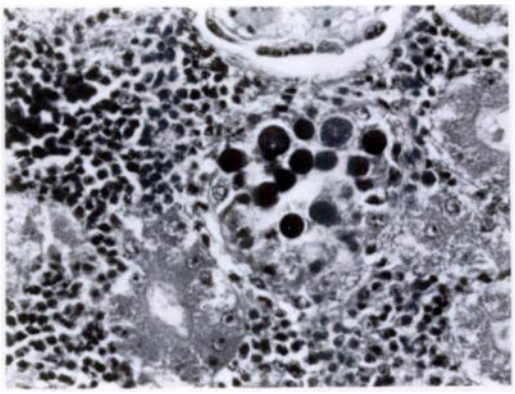

FIGURE 5. Cellular response to intracellular zygotes in the kidney of an adult Interior Canada goose (H&E, ×350).

In areas where these stages were located there was an infiltration of large numbers of macrophages and inflammatory cells into the interstitium (Fig. 5). These cells surrounded and localized oocysts in distended tubules and zygote forms in interstitial tissue and tubular cells. Such a macrophage response was observed less commonly in immature geese. Only three of the 15 infected immature geese (20%) had a significant cellular response to the presence of parasite stages in kidney tissue, whereas five of six adult geese (83.3%) had some form of macrophage infiltration in association with the infection.

An immature female Canada goose found dead at Horicon NWR was sent to the National Wildlife Health Laboratory for necropsy in November of 1979. At postmortem the goose's kidneys were swollen, pale and mottled with whitish foci on the surface. Wet smears made from one kidney contained enormous numbers of coccidan oocysts, which were identical morphologically to the oocysts found in other MVP Canada geese. The cause of death was diagnosed as massive renal coccidiosis. This is the first reported case of MVP Canada goose mortality from renal coccidiosis. The lesions associated with the presence of the oocysts in the kidney tissue were similar to those described in geese collected for our study, but much more severe. Attempts to sporulate the oocysts teased from the unfrozen kidney tissue were unsuccessful.

DISCUSSION

The recovery of the oocysts of this species of *Eimeria* from the kidney tissue and ureters of MVP Canada geese clearly establishes this parasite as a renal coccidian. However, the oocysts of this coccidium do not morphologically resemble those of *E. truncata*. The more round bottomed oocysts of the *Eimeria* sp. reported here were larger than the more ovoid *E. truncata* according to the original descriptions given by Railliet and Lucet (1890) (14–22 μm by 11.7–16 μm). Although reported ranges for *E. truncata* have been as great as 14 to 27 μm in length and 12 to 22 μm in width (Todd and Hammond, 1971), most measurements for *E. truncata* reported in the literature fall within the original group of ranges. The distinctly truncated end characteristic of oocysts of *E. truncata* was not present on the oocysts identified here.

The oocysts of this coccidium somewhat resembled the unsporulated oocysts of *E. clarkei* Hanson, Levine, and Ivens, 1957, which were collected from lesser snow geese, *Chen caerulescens caerulescens* (Linnaeus), at Winisk, Ontario. The range of measurements given for *E. clarkei* in the original description (25–30 μm by 18–21 μm; mean: 27.3 by 19.3 μm) is similar to that for the oocysts found in Canada geese, although the mean measurements were somewhat larger. However, because *E. clarkei* has been found only in fecal samples, it is unclear whether it is an enteric or renal coccidium, thus its identification remains obscure.

Greiner et al. (1981) reported finding oocysts of *E. clarkei* in fecal samples of Aleutian Canada geese (*B. c. leucopareia* (Brandt)) and gave measurements very similar to the oocysts reported here (23–

28 μm by 16–22 μm; mean: 26.4 by 18.4 μm). However, they stated that *E. clarkei* oocysts recovered in their study resembled the unidentified oocysts found in the kidneys of ducks (Wobeser, 1974; Nation and Wobeser, 1977). Oocysts found in this study have no morphologic resemblance to any renal coccidian oocysts reported from ducks.

The oocysts of *E. hermani* Farr, 1953 are also very similar to the oocysts described here in both measurements (17.5–19.5 μm by 24.3–27.6 μm) and form. Although Farr (1953) originally reported this species from the Canada goose and experimentally from the intestine of the domestic goose (*Anser anser* (Linnaeus)), there is no mention whether she examined the host's kidneys or not. Thus, there is a possibility that she could have worked with a mixed infection, mistaking the endogenous stages observed in the intestine for *E. hermani*. The oocyst wall of *E. hermani* is reported to be double-layered, however, both layers are not easily seen unless the oocyst is broken under pressure. The oocysts of the *Eimeria* reported here had only a single layer that could be observed, but none of the oocysts were crushed and examined for a second layer.

Unidentified oocysts resembling those described by Gajadhar et al. (1982) were also found in the kidneys of two of 24 lesser snow geese collected at Winisk. Though similar in appearance to oocysts from MVP Canada geese, the snow goose oocysts had several morphological differences and were not considered to be identical.

The species of coccidium was not determined in this study although its oocysts resembled several species previously described. Without data from experimental infections to standardize the morphology and measurements of the endogenous forms and sporulated oocysts of this species of *Eimeria*, attempts to identify this parasite would be speculative at best.

The actual numbers of infected birds recovered from each sample area were very close and show that the infection is not exclusive to one area of the flyway. Though specific areas where birds acquired the infection were not determined, the higher prevalence at Winisk suggested that the greatest percentage of infections in MVP Canada geese may occur during the summer months on the breeding grounds.

Walden (1961) stated that renal coccidiosis is mainly a disease of younger birds and reported a higher prevalence of infection in immature birds than in adults. Wobeser (1974) and Nation and Wobeser (1977) also reported significantly more juvenile than adult ducks were infected with renal coccidia (*Eimeria* spp.). Our data also reinforce the concept of greater susceptibility of immature birds to renal coccidian infections because significantly more immature than adult Canada geese were infected with renal coccidia. The high percentage of infected immature geese (73%) shedding oocysts also suggests that this age group may play the most significant role in the distribution of renal coccidian oocysts within this subpopulation. Few immature geese showed a cell mediated response to the infection. Most infected adult geese (5 of 6) exhibited cellular immune response probably through prior contact with the parasite.

Although the prevalence of renal coccidia in the sampled population of MVP geese was relatively low, and did not appear to affect the majority of infected geese abnormally, the pathogenicity of the parasite was demonstrated by the death of the goose from Horicon NWR. The infection can cause significant damage to renal epithelial cells, renal tubular structure, and interrupt normal kidney function and should be regarded as an important potential pathogen in this population.

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